



Evaluation of potentially toxic elements (PTEs) contamination in seawater, sediment, and sea snails (*Nerita articulata* and *Cerithidea obtusa*) from Kukup Fishing Village, Johor, Malaysia

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Received: 6 February 2025 / Accepted: 3 April 2025
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Abstract Molluscs, being highly susceptible to potentially toxic elements (PTEs) and easily accessible for human consumption, play a critical role in research on PTE pollution. This study focuses on Kukup Fishing Village in Johor, Malaysia, to investigate the levels of Cd, Cu, and Pb in seawater, sediment, and the soft tissues and shells of the gastropods *Nerita articulata* (lined nerite snail) and *Cerithidea obtusa* (obtuse horn shell). All collected samples were chemically digested before being analysed using the flame atomic absorption spectrometer (F-AAS). It is found that the PTEs are mostly accumulated in soft tissues and shells, followed by sediments and seawater, respectively. Besides, the results revealed that all study locations exhibited moderate to severe pollution, and the PTE concentrations in sea snails exceeded the thresholds set by the Malaysian Food

Regulations (1985). The mean concentrations of the studied elements in the seawater, soft tissues, and shells of two studied species were found to be in the order of $Pb > Cu > Cd$. Additionally, Pb was heavily accumulated in the soft tissues of both species: *N. articulata* (712.587–723.242 ppm) and *C. obtusa* (705.935–708.626 ppm). Compared to *N. articulata*, *C. obtusa* showed a higher capacity to accumulate Cd (3.702–4.350 ppm) and Cu (92.687–157.445 ppm), particularly in the soft tissues. The shell of *N. articulata* and the soft tissue of *C. obtusa* were identified as potential biomonitoring indicators for Cd and Cu, respectively. It is recommended that the local Malaysian authorities could strengthen environmental management, implement regular monitoring, and raise public awareness to minimise seafood consumption from polluted areas as these measures could aid to reduce pollution, protect marine ecosystems, and safeguard public health.

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Keywords Potentially toxic elements (PTEs) ·
Gastropod accumulation · *Nerita articulata* ·
Cerithidea obtusa · Seafood safety

Introduction

The exponential growth of human pollution and the rising hazard of anthropogenic pressure, which jeopardises all aquatic ecosystems, have led to a rise in marine environment pollution (Krishnan et al., 2022b;

Kumar et al., 2022; Yap & Al-Mutairi, 2022). PTE contamination of resources could have a catastrophic impact on the functioning of the ecosystem and even lead to biodiversity loss and extinction of susceptible species (Ali et al., 2019; Tovar-Sánchez et al., 2018). Since PTEs are not biodegradable, even though they are typically present in natural water bodies at concentrations below thresholds of toxicological concern, such concentrations may still cause hazards through bioaccumulation in organisms that cannot efficiently metabolise them (Ranjan & Babu, 2016). Prolonged exposure to these metals in the environment is biologically hazardous, as the pollutants have a dangerous impact on aquatic creatures, ecosystem, and human health in addition to persisting in ecosystems and contaminating the food web (Ali et al., 2019; Kapahi & Sachdeva, 2019; Martínez et al., 2010). The origin of species, their habitat environment, and their roles within marine food webs could significantly influence the bioaccumulation and excretion of pollutants in aquatic organisms (Kumar et al., 2022; Stanchev et al., 2011; Wieczorek-Dąbrowska, 2013; Tek et al., 2024). Gastropods have an ecological role as biological indicators because they are susceptible to environmental changes due to their ability to absorb minerals and deposit PTEs from their habitat environment into tissues and shells (Santhanam, 2018). Most of these PTEs are used in developing gastropod soft tissues and shells, especially the growth of shells that can reflect the impact of human activities on their ecosystems from the chemical aspects of shells (Carell et al., 1987; Freitas et al., 2016). As a result, protecting these gastropods from toxic substances is crucial because they are a vital component in food webs and the metal cycles in the aquatic system. Bioaccumulation of PTEs can be significant in terms of public health, especially when molluscs are easily accessible and consumed by humans (Ranjan & Babu, 2016).

Many biological assessment studies have focused on metal contamination, with mammals, fishes, invertebrates, and plants being the most frequently used species for biomonitoring (Wang, 2016; Zhou et al., 2008). Each group of sentinel organisms offers distinct advantages in the biological monitoring of metal contamination in aquatic environments. Monitoring and preventing PTE contamination has emerged as a significant research focus due to growing concerns about the widespread presence of PTEs in aquatic ecosystems (Ranjan & Babu, 2016; Salah-Tantawy

et al., 2024). In Malaysia, most studies have focused on the concentrations of potentially hazardous PTEs in sediment or the muscle tissue of specific mollusc species found in mangrove habitats (Ismail et al., 2004; Yap & Al-Mutairi, 2022; Yap et al., 2016). However, few studies have compared different types of sentinel organisms for assessing PTE contamination at the same site (Ismail et al., 1991; Rabaoui et al., 2014; Yusoff & Long, 2011).

Kukup Fishing Village, located in Johor, Malaysia, is a commercial fishing community primarily engaged in tourism and fish farming (Manan et al., 2011). The village has a high density of cage culture, which caused aggregation of PTE from the upstream agriculture activities (Chor et al., 2022). Besides, Kukup Fishing Village is also located near the Port of Singapore, serving as a major passage for ferries and ships. Oil spills from fishing boats, tourist boats, and vessel maintenance activities continue to degrade the marine environment, posing a serious threat to marine life (Chor et al., 2022; Manan et al., 2011). These activities have resulted in severe pollution problems, such as issues related to shipping and wastewater management (Chor et al., 2022). Although the local authorities have implemented the Waste Management and Public Cleansing Regulations 2018 in Pontian, Johor, waste disposal remains a persistent challenge for local authorities (The Star, 2022). Most waste and wastewater, including cleaning agents, construction materials, food residues, garbage, and excrement, are discharged directly into the seawater without proper treatment.

Due to the lack of information on environmental and biosafety issues, many residents of Kukup harvest seafood directly for consumption or commercial purposes without considering food safety. According to Chor et al. (2022), the estimated population of Kukup was around 1400, including approximately 79 fish farmers and 8000 cages in 2022, contributing to the local seafood market. Besides, tourism activities such as homestays and seafood restaurants make food safety a more urgent concern. The commonly available sea snails, such as *Nerita articulata* (lined nerite snail) and *Cerithidea obtusa* (obtuse horn shell), are well-recognised bioindicators for pollution detection due to their biological traits, which make them susceptible to bioaccumulation (Kumar et al., 2022; Yap & Cheng, 2013). Particularly in the rural areas of Malaysia, these two sea snail species are highly

abundant and popular in the seafood market (Yap & Al-Mutairi, 2023). According to Yap and Al-Mutairi (2023), the production of *C. obtusa* is around 78 tonnes in 2021 alone. Besides direct consumption, *C. obtusa* is also used as traditional medicine to treat some illnesses including fever, wounds, and itching (Misnan et al., 2016). As a result, to increase local awareness of food safety and environmental concerns, assessing the potentially toxic elements (PTEs) contamination in these sea snails is imperative.

Therefore, this study aimed to evaluate the concentrations of PTEs, including cadmium (Cd), copper (Cu), and lead (Pb), due to their high toxicity and environmental impact. These metals are also regulated under the Malaysian Food Regulations (1985) and were analysed in seawater, sediments, and sea snails from the Kukup Fishing Village. The study seeks to provide a new perspective on investigating PTE contamination and evaluate these sea snails' suitability as bioindicators for PTE pollution. The specific objectives of the study were to (1) determine the concentrations of Cd, Cu, and Pb in two distinct parts (shell and soft tissues) of *Nerita articulata* and *Cerithidea obtusa* in Kukup Fishing Village; (2) evaluate the relationships between PTE concentrations in *N. articulata* and *C. obtusa* with those in the surrounding seawater and sediment; and (3) assess PTE exposure and conduct a risk assessment for *N. articulata* and *C. obtusa* using various environmental monitoring indices such as the biota-sediment accumulation factor (BSAF), bioaccumulation factor (BAF), contamination factor (CF), geo-accumulation index (I_{geo}), and pollution load index (PLI).

Materials and methods

Sampling and sample pre-treatment

The present study was conducted in Kukup Fishing Village, Johor, Malaysia, in April 2023, at three stations, (a) Kukup Fishermen Jetty (Station 1: 1°19'34.8"N, 103°26'29.6"E), (b) community settlement (Station 2: 1°19'37.1"N, 103°26'32.4"E), and (c) mangrove area (Station 3: 1°19'38.7"N, 103°26'37.7"E), as shown in Fig. 1. At each station, three replicates of sediment and seawater samples were collected. For the sea snail samples, five individuals of each species, *Nerita articulata* and

Cerithidea obtusa, were harvested, with sizes ranging from 3.5 to 4.0 cm and 2.0 to 3.0 cm, respectively. However, due to force majeure in Station 1, *C. obtusa* was not able to be collected. After collection, similar-sized samples were selected for replicates to minimise bias in PTE content due to size variations. A total of *N. articulata* ($n = 15$) and *C. obtusa* ($n = 10$) samples were separated, rinsed with seawater, and kept for at least 12 h for defecation (Amin et al., 2013). Surface sediments were collected at a depth of no more than 5.0 cm using a shovel and placed in polypropylene (PP) sampling bags. Seawater samples were collected in pre-cleaned 250-mL high-density polyethylene (HDPE) bottles, and the physico-chemical parameters of seawater, including temperature and salinity, were measured and recorded during sampling. All collected samples were stored in ice boxes and subsequently preserved at -20°C in the laboratory for further analysis (Sun et al., 2020).

The sea snail samples were rinsed with ultrapure water and separated into shells and soft tissues (Amin et al., 2013), followed by drying in an oven at 60°C until a constant weight was achieved (Krishnan et al., 2022a). Sediment samples were also dried in an oven at 60°C for at least 3 days until reaching a constant weight. Both dried samples were homogenised into powder using a mortar and pestle and then sieved through a 63- μm stainless steel mesh (Krishnan et al., 2022a). Water samples were filtered using a Whatman 42 filter paper (Sun et al., 2020).

Sample and chemical analyses

To avoid contamination, all glassware and equipment were cleaned with 10% nitric acid (HNO_3) and rinsed thoroughly with ultrapure water (Ranjan & Babu, 2016). Quality control measures included using procedural blanks for seawater and sea snail shell samples. For sediments and soft tissues, the accuracy of the analysis was confirmed by testing certified reference materials (CRMs). Sediment analysis used BCR-142R-0924 (light sandy soil) as the reference material, while soft tissue analysis used mussel tissues (ERM-CE278k-1069). The recovery rates for PTEs in the CRM soft tissue samples were satisfactory: 102.78% for lead (Pb), 95.95% for cadmium (Cd), and 120.52% for copper (Cu). Similarly, recovery rates for the CRM sediment samples were 83.64%, 85.29%, and 91.59% for Pb, Cd, and Cu, respectively.

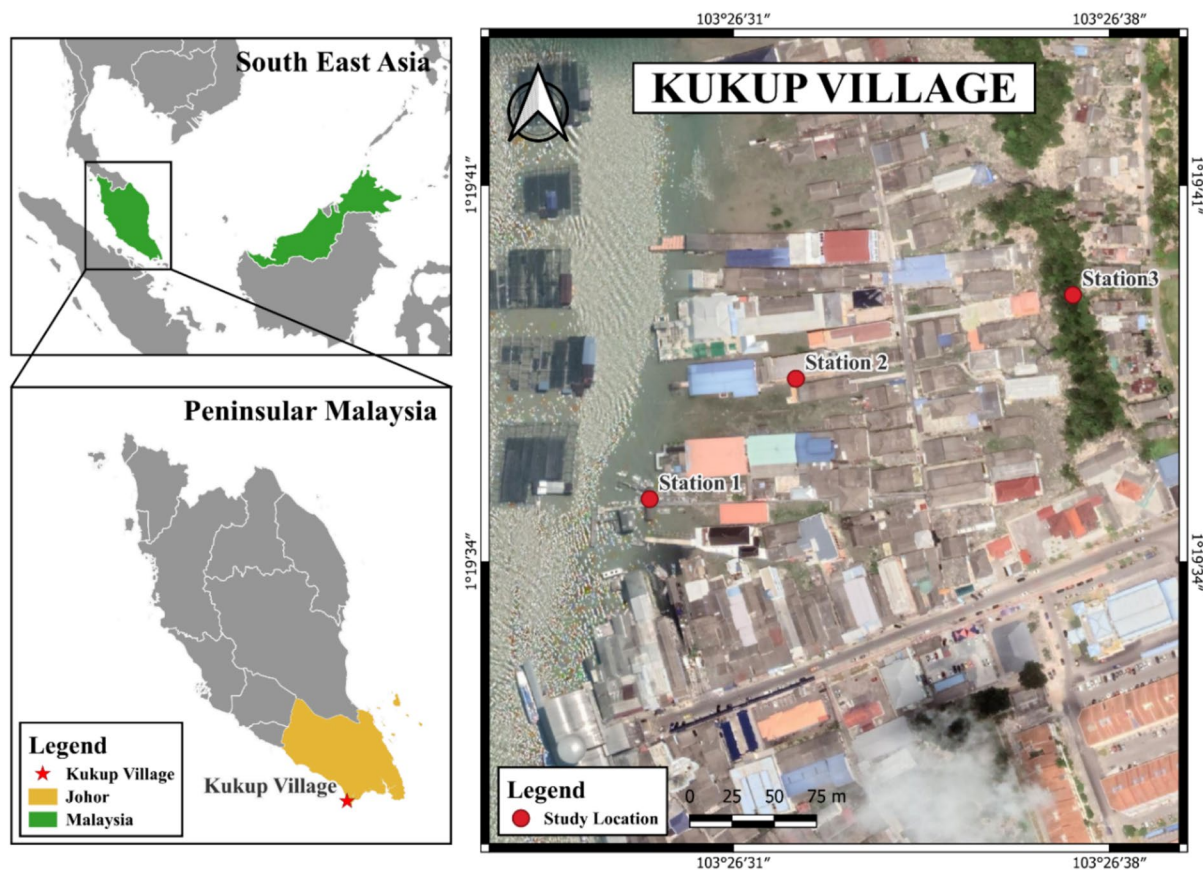


Fig. 1 Sampling stations in Kukup Village, Johor, Malaysia

These results confirm that the methods used in this study were reliable and accurate for measuring PTE concentrations in sediments and soft tissues.

Sea snail

The acid digestion of sea snails was divided into two parts, the soft tissues and shells. For the acid extraction of soft tissues, the method was adapted from Idera et al. (2015) and US EPA (1996). 0.1 g of dried samples was digested in 5 mL of concentrated nitric acid (69% HNO_3) and 5 mL of concentrated sulphuric acid (98% H_2SO_4). The samples were then heated at 70 °C for half an hour after the reaction of samples with HNO_3 and H_2SO_4 had ceased. Another 10 mL of 69% HNO_3 was added after the solution had been allowed to cool for 15 min. The samples were then heated at 95 °C without boiling for at least 3 h. After allowing the samples to cool for an hour, 2 mL of

ultrapure water and 3 mL of hydrogen peroxide (30% H_2O_2) were added and then placed aside until effervescence ceased. For the shells, 0.1 g of dried samples was digested in 5 mL of 69% HNO_3 , heated at 95 °C without boiling, and refluxed for 30 min. The samples were allowed to cool aside for 15 min, and then, 2.5 mL of perchloric acid (70% HClO_4) was added and heated for 25 min or until dense white fumes were observed (Hseu, 2004).

Sediment

The acid extraction of sediment was adapted from US EPA (1996), where 0.1 g of each dried sample was digested with 10 mL of 69% HNO_3 and ultrapure water in a ratio of 1:1, heated at 95 °C without boiling, and refluxed for 15 min. The solution was allowed to cool for 15 min, and 5 mL of 69% HNO_3 was added into a conical flask and refluxed for 30

min. This step was repeated until no brown fumes were observed. The solution was heated for 2 h without boiling at 95 °C and then allowed to cool for 10 min. Two millilitres of ultrapure water and 3 mL of 30% H₂O₂ were added and heated until effervescence residues and cooled for approximately 5 min. Then, 2 mL of 30% H₂O₂ was added and heated for 5 min until minimal effervescence was observed. The solution was heated for 2 h without boiling at 95 °C, or until the volume was reduced to approximately 5 mL. Ten millilitres of hydrochloric acid (37% HCl) was added into a conical flask, heated for an hour, and refluxed for half an hour. The solution was cooled for 15 min and then 2 mL of 30% H₂O₂ was added.

Seawater

The acid extraction of seawater was adapted from the Ministry of Environmental Protection of the People's Republic of China (2014). Twenty-five millilitres of filtered seawater was measured and transferred to a 100 mL conical flask. Two millilitres of concentrated 69% HNO₃ and 6 mL of 37% HCl were added into the conical flask and then heated at 200 °C until the solution became clear and transparent or until the remaining volume was approximately 7 to 10 mL. All digested solution was allowed to cool overnight in a fume hood. Then, the digested samples were filtered through a Whatman 42 filter paper and diluted with ultrapure water to a final volume of 50 mL.

All of the digested samples were transferred to specimen bottle and proceeded for further metal concentration detection using the flame atomic absorption spectrometer (F-AAS) (Thermo Scientific iCE 3500 Series).

Data and statistical analyses

The biota-sediment accumulation factor (BSAF) is defined as the ratio of the concentration of an element in biota to the concentration of the same element in sediment (Saroop & Tamchos, 2021). To estimate the BSAF for the studied PTEs, the following equation was applied:

$$BSAF = \frac{C_X}{C_S}, \quad (1)$$

where C_X represents the mean metal concentration in the sea snail (shell or soft tissue) whereas C_S represents the mean metal concentration in the sediment (Szefer et al., 1999). The concentrator is considered a deconcentrator when the BSAF value is less than 1, or a microconcentrator when the BSAF value is between 1 and 2. When the BSAF value is greater than 2, the concentrator is considered to be a macroconcentrator (Dallinger, 1993).

The bioaccumulation factor (BAF) describes the typical long-term bioaccumulation of metals in aquatic species and is calculated using the formula:

$$BAF = \frac{C_X}{C_W}, \quad (2)$$

where C_X and C_W denote the mean metal concentration in the sea snail and seawater, respectively (USEPA, 2000).

To evaluate the quantity of metals introduced into the sediment due to anthropogenic activities, the contamination factor (CF) reveals the level of metal contamination in sediment (Ahmed et al., 2016). The CF is calculated using the following equation:

$$CF = \frac{C_N}{B_N}, \quad (3)$$

where C_N represents the metal concentration in the sample, and B_N represents the background metal concentration in typical shale (Hakanson, 1980; Krishnan et al., 2022a). In this study, the world average shale data from Rahman et al. (2022) and Turekian and Wedepohl (1961) were used as references for metal background values (0.3 ppm for Cd, 45 ppm for Cu, and 20 ppm for Pb), as regional background levels for Cd, Cu, and Pb are currently unavailable (Ong et al., 2013).

To determine the degree of PTE pollution and sediment quality, the pollution load index (PLI) was calculated using the formula (Banerjee & Gupta, 2012; Islam et al., 2015; Radomirović et al., 2020):

$$PLI = \sqrt[n]{(CF_1 \times CF_2 \dots \times CF_n)}, \quad (4)$$

where CF represents the contamination factor and n is the number of metals.

For identifying metal pollution in aquatic sediments, the geo-accumulation index (I_{geo}) was calculated using the equation:

$$I_{\text{geo}} = \log_2 \left(\frac{C_N}{1.5B_N} \right), \quad (5)$$

where C_N denotes the metal concentration in the sediment sample, B_N denotes the geochemical background concentration of the average continental shale, and a factor of 1.5 represents the correction for the background matrix fluctuations, accounting for potential changes due to minimal anthropogenic and lithological effects and comparing contemporary metal concentrations with the pre-industrial levels (Radomirović et al., 2020). Similarly, in this study, the world average shale data from Rahman et al. (2022) and Turekian and Wedepohl (1961) were used as references for metal background values (0.3 ppm for Cd, 45 ppm for Cu, and 20 ppm for Pb), as regional background levels for Cd, Cu, and Pb are currently unavailable (Ong et al., 2013).

The Jarque–Bera test was employed to determine the normal distribution of the collected data. One-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test, was used to evaluate the significance of PTE concentrations with respect to different study locations, species, and sample types separately at the 95% statistical confidence level. Two-way and three-way

ANOVA were applied to investigate the effect of species and study location on the variation of PTE concentrations in sea snails (Szefer et al., 1999). Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 27 (Ranjan & Babu, 2016). Pearson's correlation was used to examine the relationships between PTE concentrations in sediment, seawater, soft tissues, and shells of the two studied species.

Results and discussion

PTE accumulation in sea snails, sediment, and seawater

The concentrations of the studied elements in soft tissues and shells of *N. articulata* and *C. obtusa* were found to be in the order of Pb > Cu > Cd (Table 1). Compared to previous studies, it was found that for *N. articulata* soft tissue, the metal concentration ranges (ppm, dry weight) for Pb (712.59–723.24), Cd (1.92–2.57), and Cu (16.17–24.39) fell within the ranges reported in previous studies as shown in Table 2 (Yap et al., 2009). However, for *C. obtusa* soft tissues, only Cd concentrations (ppm) (3.70–4.35)

Table 1 Metal concentration (ppm, dry weight) in the soft tissue and shell of *N. articulata* and *C. obtusa*, as well as sediment and seawater from Kukup Fishing Village

Species (sample type)		Site	Concentration ±SD (ppm, dry weight)		
			Pb	Cd	Cu
<i>N. articulata</i>	Shell	Station 1	46.988 ± 5.993	4.919 ± 0.587	23.557 ± 1.879
		Station 2	49.171 ± 11.657	5.402 ± 0.792	21.626 ± 3.168
		Station 3	53.007 ± 9.554	4.535 ± 0.563	20.489 ± 2.842
	Soft tissue	Station 1	712.587 ± 14.400	2.568 ± 0.937	16.173 ± 1.954
		Station 2	723.242 ± 13.663	2.349 ± 0.825	24.391 ± 2.343
		Station 3	714.796 ± 12.229	1.916 ± 0.521	20.327 ± 0.415
	Shell	Station 1	NA	NA	NA
		Station 2	39.195 ± 6.949	4.874 ± 0.366	20.130 ± 3.103
		Station 3	32.921 ± 7.799	3.717 ± 0.156	20.233 ± 1.058
<i>C. obtusa</i>	Soft tissue	Station 1	NA	NA	NA
		Station 2	708.626 ± 11.308	4.350 ± 0.304	157.445 ± 21.278
		Station 3	705.935 ± 12.651	3.702 ± 0.456	92.687 ± 21.996
	Sediment	Station 1	22.688 ± 4.876	1.994 ± 0.085	28.116 ± 0.716
		Station 2	19.624 ± 6.357	2.365 ± 0.166	34.748 ± 4.708
		Station 3	23.906 ± 5.347	4.168 ± 0.584	32.446 ± 4.251
	Seawater	Station 1	0.077 ± 0.012	0.022 ± 0.001	0.067 ± 0.008
		Station 2	0.110 ± 0.086	0.022 ± 0.002	0.061 ± 0.005
		Station 3	0.110 ± 0.064	0.024 ± 0.001	0.066 ± 0.004

SD standard deviation, NA not available

Table 2 A comparison of recent reported studies on the metal concentration (ppm, dry weight) of Pb, Cd, and Cu in the soft tissues and shells of *N. articulata* and *C. obtusa*, as well as sediment and seawater in Malaysia

Sample	Location	Pb (ppm)	Cd (ppm)	Cu (ppm)	Reference
Malaysian Food Regulation		2	1	30	Malaysian Food Regulation (1985)
<i>N. articulata</i>					
Shell	Kukup Fishing Village, Johor	46.99–53.01	4.54–5.40	20.49–23.56	Present study
	Kukup, Johor	59.41	4.57	5.68	Amin et al. (2013)
	Janggut river, Selangor	2.86	3.13	1.4	Yap and Cheng (2013)
	West Coast of Peninsular Malaysia	22.30–84.40	1.60–6.40	1.30–11.10	Yap et al. (2009)
	Peninsular Malaysia	48.86	3.15	5.59	Yap and Cheng (2009)
	Peninsular Malaysia	48,860	3150	5590	Adnan and Ismail (2014)
Soft tissue	Kukup Fishing Village, Johor	712.59–723.24	1.92–2.57	16.17–24.39	Present study
	Kukup, Johor	36.39	1.03	27.63	Amin et al. (2013)
	Janggut river, Selangor	5.69–67.29	1.10–6.83	4.56–19.12	Yap and Cheng (2013)
	West Coast of Peninsular Malaysia	1.40–1251	2.8	11.50–25.20	Yap et al. (2009)
	Peninsular Malaysia	92,720	1030	2650	Adnan and Ismail (2014)
	Peninsular Malaysia	1.03–50.47	0.69–6.25	6.33–25.82	Cheng and Yap (2015)
<i>C. obtusa</i>					
Shell	Kukup Fishing Village, Johor	32.92–39.20	3.72–4.87	20.13–20.23	Present study
	Sepang river, Selangor	NA	0.311–0.336	1.73–2.50	Kumar et al. (2022)
	Bako, Sarawak	56.2	5.1	11.9	Edward et al. (2010)
	Sematan, Sarawak	53.2	5.4	11	Edward et al. (2010)
	Deralik, Sitiawan	63.8	4.4	8.6	Edward et al. (2010)
Soft tissue	Kukup Fishing Village, Johor	705.94–708.63	3.70–4.35	92.69–157.45	Present study
	Sepang river, Selangor	NA	0.64–0.90	90.59–103.88	Kumar et al. (2022)
	Peninsular Malaysia	3.75–55.90	0.11–9.65	47.60–147.00	Yap and Al-Mutairi (2023)
Sediment	Kukup Fishing Village, Johor	19.62–23.91	1.99–4.17	28.12–34.75	Present study
	Janggut river, Selangor	22.70–24.30	1.95–2.31	2.94–3.55	Yap and Cheng (2013)
	Juru river, Penang	27.64–45.25	0.57–0.70	NA	Krishnan et al. (2022a)
	Port Klang, Selangor	31.30–104.87	0.14–2.10	8.60–57.01	Sany et al. (2013)
	Port Klang, Selangor	8.16–16.77	NA	1.89–6.13	Haris et al. (2020)
	Peninsular Malaysia	25.36–172.57	1.11–2.00	5.59–28.71	Cheng and Yap (2015)
Seawater	Kukup Fishing Village, Johor	0.077–0.110	0.022–0.024	0.061–0.067	Present study
	Southern Peninsular Malaysia	0.018–0.377	0.013–0.147	0.024–0.143	Yap et al. (2011)
	Port Klang, Selangor	0.00164–0.0071	0.00033–0.00107	0.00167–0.00529	Sany et al. (2012)

NA not available

were aligned with the findings reported by Yap and Al-Mutairi (2023), whereas the concentration ranges (ppm) for Pb (705.94–708.63) and Cu (92.69–157.45) exceeded the ranges reported in previous studies (Table 2) (Kumar et al., 2022; Yap & Al-Mutairi, 2023). As for shell of both species, the concentration (ppm) ranges of Pb (32.92 ± 7.80 to 53.01 ± 9.55), Cd (3.72 ± 0.16 to 5.40 ± 0.79), and Cu (16.17 ± 1.95 to 24.39 ± 2.34) were within the range of the previous study, except for the Cu concentration in the shell of *C. obtusa* (Adnan & Ismail, 2014; Amin et al., 2013; Edward et al., 2010; Yap & Cheng, 2009, 2013; Yap et al., 2009).

In the sediment sampled from the three study locations, the concentrations (ppm, dry weight) of PTEs decreased in the following order: Cu (28.12 ± 0.72 to 34.75 ± 4.71) > Pb (19.62 ± 6.36 to 23.91 ± 5.35) > Cd (1.99 ± 0.09 to 4.17 ± 0.58) (Table 1). The concentrations of Pb and Cu in the sediment fell within the ranges reported from previous studies, except for Cd, which exceeded the reported (Table 2). In terms of seawater samples, the concentrations (ppm, dry weight) of the studied elements were found to be in the order of Pb (0.08 ± 0.01 to 0.11 ± 0.09) > Cu (0.02 ± 0.01 to 0.02 ± 0.01) > Cd (0.06 ± 0.01 to 0.07 ± 0.01), which were within the ranges reported in previous studies (Table 2) (Yap et al., 2009, 2011).

Comparing data that were from Kukup over time (Amin et al., 2013; Espejo et al., 2019; Javed et al., 2019), specifically in the soft tissues of *N. articulata*, revealed a significant increase in Pb and Cd concentrations (Table 2). The substantial increase of Pb and Cd concentration in Kukup Fishing Village indicates the presence of potential anthropogenic sources such as power plant and plastic industries, shipping activities, untreated sewage water, and the combustion of charcoal (Chor et al., 2022; Manan et al., 2011). Table 2 shows all the studied element concentrations in this study exceeded the concentrations set by the Malaysian Food Regulations (1985). The exceeding amount of PTEs calls for extra attention from the relevant authority and an increase in local awareness regarding the consumption of these sea snails in terms of food safety.

In the case of species, a significant difference was found between *N. articulata* and *C. obtusa* in terms of Cd and Cu concentrations. The differences indicate that both studied species have varying capabilities to accumulate Cd and Cu, with *C. obtusa* showing a

Table 3 Mean concentration (ppm, dry weight) of Pb, Cd, and Cu against different sea snail species, sampling sites, and sample types of *N. articulata* and *C. obtusa* from Kukup Fishing Village

	Pb (ppm)	Cd (ppm)	Cu (ppm)
Species			
<i>N. articulata</i>	383.298 ^a	3.331 ^a	21.136 ^a
<i>C. obtusa</i>	371.669 ^a	4.161 ^b	72.624 ^b
Sites			
Station 1	195.585 ^a	2.070 ^a	16.560 ^a
Station 2	256.661 ^a	3.083 ^a	42.877 ^a
Station 3	255.112 ^a	3.074 ^a	31.552 ^a
Sample types			
Sediment	22.073 ^a	2.842 ^a	31.770 ^{ab}
Shell	44.256 ^b	4.348 ^b	21.258 ^a
Soft tissue	713.037 ^c	2.977 ^a	62.205 ^b
Seawater	0.099 ^d	0.023 ^c	0.065 ^a

The same letter shows no significant difference as indicated by a post hoc statistical test with a 95% confidence level.

Table 4 Mean concentration (ppm, dry weight) of Pb, Cd, and Cu within sampling sites of *N. articulata* and *C. obtusa* from Kukup Fishing Village. The same letter shows no significant difference as indicated by a post hoc statistical test with a 95% confidence level

Species—site	Pb (ppm)	Cd (ppm)	Cu (ppm)
<i>N. articulata</i>			
Station 1	379.788 ^a	3.133 ^a	19.028 ^a
Station 2	386.206 ^a	3.442 ^a	22.440 ^a
Station 3	383.902 ^a	3.417 ^a	21.942 ^a
<i>C. obtusa</i>			
Station 1	NA	NA	NA
Station 2	373.910 ^a	4.612 ^a	88.788 ^a
Station 3	369.427 ^a	3.710 ^a	56.460 ^a

NA not available

higher ability to accumulate Cd and Cu elements than *N. articulata*. The reason for this type of accumulation could be the different diets, as *C. obtusa* primarily feeds on sediment while *N. articulata* mainly consumes microalgae (Thanh-Nho et al., 2019).

However, no significant differences were observed among the three sampling sites for Pb, Cd, and Cu (Tables 3 and 4), suggesting that the sampling sites do not influence the differences in the accumulation of these elements. As indicated in Table 3, it was found that there are significant differences ($p < 0.05$)

between sediment, shell, soft tissue, and seawater in terms of Pb concentration. In contrast, no such significant difference was found for Cd and Cu. The reason causing such difference may have something to do with the varying entrance pathways of different elements into marine ecosystems, dietary intake, and feeding behaviours, among others (King et al., 2004; Kumar et al., 2022).

Regarding Cd concentration, no significant difference was observed between sediment and soft tissues, suggesting that the soft tissues are ineffective at accumulating Cd from the sediment. The reason that causes such phenomena could be the concentration of PTE in soft tissue which is greatly affected by the reproduction and biological status of gastropods, whereas for shell, it is not significantly influenced (Yap et al., 2003). A significant difference was found between soft tissues and shells regarding Cu concentration, indicating that soft tissues have a higher ability to accumulate Cu as this finding is consistent with Shirneshan and Bakhtiari's (2012) and Yap and Cheng's (2013) studies. Soft tissues of both studied species also showed a significant difference compared to seawater for Cu concentration, revealing that both *N. articulata* and *C. obtusa* can accumulate significant levels of Cu in their soft tissues. Table 5 shows

Table 5 Mean concentration (ppm, dry weight) of Pb, Cd, and Cu between sites and sample types of *N. articulata* and *C. obtusa* from Kukup Fishing Village

Site—sample type	Pb (ppm)	Cd (ppm)	Cu (ppm)
Station 1			
Sediment	22.688 ^a	1.994 ^a	28.116 ^a
Shell	46.988 ^a	3.698 ^a	21.882 ^a
Soft tissue	712.587 ^a	2.568 ^a	16.173 ^a
Seawater	0.077 ^a	0.021 ^a	0.067 ^a
Station 2			
Sediment	19.624 ^b	2.365 ^b	34.748 ^a
Shell	44.183 ^b	4.705 ^b	20.309 ^a
Soft tissue	715.934 ^b	3.349 ^b	90.918 ^a
Seawater	0.110 ^b	0.022 ^b	0.061 ^a
Station 3			
Sediment	23.906 ^b	4.168 ^b	32.446 ^a
Shell	42.964 ^b	4.318 ^b	21.895 ^a
Soft tissue	710.365 ^b	2.809 ^b	56.507 ^a
Seawater	0.110 ^b	0.024 ^b	0.066 ^a

The same letter shows no significant difference as indicated by a post hoc statistical test with a 95% confidence level.

significant differences in Pb and Cd between sample types and sampling sites by comparing all studied locations. The absence of significant differences between Stations 2 and 3 suggests that these areas exhibited similar Pb and Cd contamination levels, indicating shared sources or comparable environmental conditions affecting Pb and Cd accumulation. Nevertheless, Pb concentrations show no significant differences across stations and species, while Cd and Cu vary, with Station 2 soft tissues in *C. obtusa* having notably higher Cu (157.445 ppm) as shown in Table 6.

Biota-sediment accumulation factor (BSAF) and bioaccumulation factor (BAF)

BSAF was used to assess the PTE exposure and examine the relationship between PTE concentrations in the two studied sea snail species and the nearby sediment. In this study, the BSAF in the soft tissues and shells of *N. articulata* followed Pb > Cd > Cu, whereas Pb > Cu > Cd for the soft tissues and shells of *C. obtusa*. Pb exhibited the highest BSAF among

Table 6 Mean concentration (ppm, dry weight) of Pb, Cd, and Cu between different sites, sample types, and species (shell and soft tissue) of *N. articulata* and *C. obtusa* from Kukup Fishing Village

Site—sample type—species		Pb (ppm)	Cd (ppm)	Cu (ppm)
Station 1				
Shell	<i>N. articulata</i>	46.988 ^a	3.698 ^{ab}	21.882 ^a
	<i>C. obtusa</i>	NA	NA	NA
Soft tissue	<i>N. articulata</i>	712.587 ^a	2.568 ^{ab}	16.173 ^a
	<i>C. obtusa</i>	NA	NA	NA
Station 2				
Shell	<i>N. articulata</i>	49.170 ^a	4.535 ^b	20.489 ^b
	<i>C. obtusa</i>	39.195 ^a	4.874 ^b	20.130 ^b
Soft tissue	<i>N. articulata</i>	723.242 ^a	2.349 ^b	24.391 ^b
	<i>C. obtusa</i>	708.626 ^a	4.350 ^b	157.445 ^b
Station 3				
Shell	<i>N. articulata</i>	53.007 ^a	4.919 ^a	23.557 ^{ab}
	<i>C. obtusa</i>	32.921 ^a	3.717 ^a	20.233 ^{ab}
Soft tissue	<i>N. articulata</i>	714.796 ^a	1.916 ^a	20.327 ^{ab}
	<i>C. obtusa</i>	705.934 ^a	3.702 ^a	92.687 ^{ab}

The same letter shows no significant difference as indicated by a post hoc statistical test with a 95% confidence level.

NA not available

all the studied PTEs, especially for the soft tissues of both studied species ($BSAF > 2$), except for the shells of *C. obtusa* ($1 < BSAF < 2$), indicating the high accumulation ability of Pb for both species (Table 7). Similarly, Table 1 corresponds to this result, comparing the Pb concentration in soft tissue with sediments for both studied species; the Pb concentration in soft tissue was 32 times higher than in sediments, whereas the shells were slightly lower (by two- to threefold), suggesting that the soft tissues have a much greater accumulation capacity to remove Pb from the environment (Kumar et al., 2022).

Besides, the soft tissues of *C. obtusa* had a greater BSAF value for Cu ($BSAF = 3.694$) than in the shells ($BSAF = 0.602$) (Table 7). According to the BSAF

classification proposed by Dallinger (1993), the soft tissues of *C. obtusa* were found to act as macroconcentrators for Cu, which is similar to the findings done by Kumar et al. (2022), indicating that the soft tissues of *C. obtusa* have a strong tendency of collecting Cu from sediments and hard to release them (Kobkeathawin et al., 2021). Similarly, the soft tissue of *C. obtusa* ($BSAF = 1.364$) was identified as a microconcentrator for Cd. Also, according to the classification theory, the soft tissues of *N. articulata* were identified as a deconcentrator for Cu and Cd.

However, despite the fact that the BSAF value for the soft tissues of *N. articulata* and *C. obtusa* was not ideal, the shells of *N. articulata* and *C. obtusa* show greater BSAF values than in the soft tissues, revealing

Table 7 Biota-sediment accumulation factor (BSAF) and bioaccumulation factor (BAF) for Pb, Cd, and Cu in the shell and soft tissue of *N. articulata* and *C. obtusa* from Kukup Fishing Village

Species	Sample	Site	Pb	Cd	Cu
Biota-sediment accumulation factor (BSAF)					
<i>N. articulata</i>	Shell	Station 1	2.071	2.467	0.838
		Station 2	2.506	2.284	0.622
		Station 3	2.217	1.088	0.631
		Mean $\pm$ SD	2.265 $\pm$ 0.221	1.946 $\pm$ 0.749	0.697 $\pm$ 0.122
	Soft tissue	Station 1	31.408	1.288	0.575
		Station 2	36.855	0.993	0.702
		Station 3	29.9	0.46	0.626
		Mean $\pm$ SD	32.721 $\pm$ 3.659	0.914 $\pm$ 0.420	0.634 $\pm$ 0.064
<i>C. obtusa</i>	Shell	Station 1	NA	NA	NA
		Station 2	1.997	2.061	0.579
		Station 3	1.377	0.892	0.624
		Mean $\pm$ SD	1.687 $\pm$ 0.438	1.477 $\pm$ 0.827	0.602 $\pm$ 0.032
	Soft tissue	Station 1	NA	NA	NA
		Station 2	36.11	1.839	4.531
		Station 3	29.53	0.888	2.857
		Mean $\pm$ SD	32.820 $\pm$ 4.653	1.364 $\pm$ 0.672	3.694 $\pm$ 1.184
Bioaccumulation factor (BAF)					
<i>N. articulata</i>	Shell	Station 1	610.234	223.591	351.597
		Station 2	447.009	245.545	354.525
		Station 3	481.882	188.958	310.439
	Soft tissue	Station 1	9254.377	116.727	241.388
		Station 2	6574.927	106.773	399.852
		Station 3	6498.145	79.833	307.985
<i>C. obtusa</i>	Shell	Station 1	NA	NA	NA
		Station 2	356.318	221.545	330
		Station 3	299.282	154.875	306.561
	Soft tissue	Station 1	NA	NA	NA
		Station 2	6442.055	197.727	2581.066
		Station 3	6417.591	154.25	1404.348

SD standard deviation, NA not available

a greater tendency for Cd to accumulate in the shells. The shells of both studied species were observed to function as microconcentrators for Cd but as deconcentrators for Cu.

Soft tissues exhibited higher BSAF values for Pb and Cu compared to the shells for the two studied species and vice versa for Cd. The concentrations of Cu in the sediment were found to be relatively high in the studied areas compared to the biota, indicating that the sediment has a more remarkable ability to accumulate Cu than the sea snails. From the perspective of soft tissues and shells, soft tissues were found to be ideal bioindicators for Pb and Cu (Yap et al., 2009), while the shell was proven to be a potential bioindicator for Cd, which is consistent with Adnan and Ismail's (2014) and Kumar et al.'s (2022) studies. Among both studied species, *N. articulata* was proven to be a potential bioindicator for Pb (17.493 ± 16.842) and Cd (1.43 ± 0.784), with higher mean BSAF values of soft tissues and shell (Yap & Cheng, 2009). At the same time, *C. obtusa* was considered a potential bioindicator for Cu, with a mean BSAF of 2.148 ± 1.912 , which aligned with findings from Yap and Al-Mutairi (2023).

BAF values for a particular element can be extensive. This wide range is attributed to the complex bioaccumulation processes resulting from the absorption and retention of metals in organisms, which are mainly influenced by biological traits of organisms and environmental factors, respectively (Karlsson et al., 2002).

The BAF was used to assess the transfer of PTEs from seawater into the soft tissues and shells of *N. articulata* and *C. obtusa*. According to Table 7, the BAF values for all examined PTEs in both species followed this order: Pb > Cu > Cd. Based on the classification system established by Arnot and Gobas (2006), the BAF values for Pb in the shells of both species were below 1000, indicating a relatively low probability of Pb accumulation. However, the soft tissues of *N. articulata* and *C. obtusa* exhibited extremely high Pb accumulation potential (BAF > 5000), suggesting that soft tissues are more prone to Pb accumulation than shells. Furthermore, both species showed minimal potential for Cd accumulation, indicating a weak tendency to absorb Cd from seawater. Additionally, neither the soft tissues nor the shells of both species demonstrated a significant likelihood of Cu accumulation, except for the soft tissues of *C.*

obtusa, where the BAF values ($1000 < \text{BAF} < 5000$) indicated a moderate and gradual Cu accumulation, possibly through the food chain (Swaleh et al., 2016).

Ecological risk assessments

Contamination factor (CF), pollution load index (PLI), and geo-accumulation index (I_{geo}) were the environmental indices used to monitor and determine the degree of sediment contamination. In this study, world average shale data from Rahman et al. (2022) and Turekian and Wedepohl (1961) were used as a reference for metal background values in the CF and I_{geo} equations, as the regional background levels for Pb, Cd, and Cu were unavailable currently (Ong et al., 2013). As evident from Table 8, all study sites exhibited high Cd contamination ($\text{CF} > 6$) while showing low Cu contamination ($\text{CF} < 1$) based on the classification proposed by Hakanson (1980). Stations 1 and 3 were classified as moderate Pb contamination ($1 \leq \text{CF} < 3$), while Station 2 was categorised as having a low contamination level ($\text{CF} < 1$). The data analysis from Table 8 revealed the following order of CF values in sediment samples of this study: Cd > Pb > Cu. High levels of Cd were observed at the study site,

Table 8 Contamination factor (CF), geo-accumulation index (I_{geo}), and pollution load index (PLI) across all studied locations at Kukup Fishing Village, Johor

	Station 1	Station 2	Station 3	Mean + SD
Contamination factor (CF)				
Pb	1.134 ^b	0.98 ^a	1.195 ^b	1.103 ^b ± 0.111
Cd	6.647 ^c	7.883 ^c	13.893 ^c	9.474 ^c ± 3.876
Cu	0.625 ^a	0.772 ^a	0.772 ^a	0.723 ^a ± 0.085
Geo-accumulation index (I_{geo}) for sediment				
Pb	− 0.403 [^]	− 0.612 [^]	− 0.328 [^]	− 0.448 [^] ± 0.147
Cd	2.148 [*]	2.394 [*]	3.211 [*]	2.584 [*] ± 0.556
Cu	− 1.263 [^]	− 0.958 [^]	− 1.057 [^]	− 1.092 [^] ± 0.156
Pollution load index (PLI)				
	1.676 [#]	1.814 [#]	2.288 [§]	1.926 [#] ± 0.321

^aLow contamination

^bModerate contamination

^cHigh contamination

[^]Uncontaminated sediment

^{*}Moderate to heavy sediment contamination

[#]Moderate pollution

[§]Heavy pollution

and these elevated levels could be attributed to various anthropogenic activities occurring nearby, such as construction site waste, sewage waste, and other related factors (Javed et al., 2019; Joya-Barrero et al., 2023). Regarding Pb, the high levels observed in the study area can be attributed to anthropogenic activities of coal combustion in Kukup (Gao et al., 2018).

PLI is an environmental indicator for comparing the pollution status of various sites by evaluating a specific research site's PTE pollution level (Rahman et al., 2022). Based on the classification proposed by Banerjee and Gupta (2012), the PTE pollution in Station 3 is classified as severe ($2 \leq \text{PLI} < 3$), while Stations 1 and 2 are considered moderately polluted ($1 \leq \text{PLI} \leq 2$). These findings indicate that the study site experiences PTE pollution.

I_{geo} was also applied to evaluate the contamination level of PTEs in the studied sediments. The results indicated that the quality of the sediments in the study area was not contaminated with Pb and Cu ($I_{\text{geo}} \leq 0$) according to the classification proposed by Barbieri (2016). However, the sediments in Station 3 exhibited heavy contamination for Cd ($3 < I_{\text{geo}} < 4$), while Stations 1 and 2 showed moderate to heavy contamination ($2 < I_{\text{geo}} < 3$). The analysis of environmental indicators revealed that the sediments from all three sampling sites exhibited high contamination levels of Cd and moderate contamination levels of Pb, which correspond to the finding CF evaluation. Due to their bioavailability and mobility in the aquatic ecosystem, Pb and Cd should receive special attention as they have the potential to pose significant ecological risks that may require specific management and monitoring measures (Devi & Bhattacharyya, 2018; Rashid et al., 2023).

Correlations among metal concentrations in sediment, seawater, as well as the shell and soft tissue of *N. articulata* and *C. obtusa*

A Pearson correlation test was applied to determine the extent to which PTE concentrations in sediment, seawater, soft tissues, and shells of the two studied species were correlated. For *N. articulata*, between soft tissue and sediment, weak and negative correlations were observed with Pb and Cd, yet a positive correlation was found with Cu ($r = 0.758$, $p < 0.05$) (Table 9). The correlation of Cu concentrations in soft tissue and sediments demonstrates the

Table 9 Correlations among the metal concentrations in sediment, seawater, as well as the shell and soft tissue of *N. articulata* and *C. obtusa* at Kukup Fishing Village

Correlation		Shell	Soft tissue	Sediment	Seawater
<i>N. articulata</i>					
Pb					
Shell	1				
Soft tissue	0.658	1			
Sediment	− 0.233	− 0.418	1		
Seawater	0.923 ^{ab}	0.608	− 0.302	1	
Cd					
Shell	1				
Soft tissue	− 0.539	1			
Sediment	0.572	− 0.273	1		
Seawater	0.244	− 0.565	0.645	1	
Cu					
Shell	1				
Soft tissue	− 0.334	1			
Sediment	− 0.096	0.758 ^{ac}	1		
Seawater	0.652	− 0.459	− 0.069	1	
<i>C. obtusa</i>					
Pb					
Shell	1				
Soft tissue	− 0.414	1			
Sediment	− 0.082	− 0.093	1		
Seawater	− 0.108	− 0.715 ^{ac}	− 0.302	1	
Cd					
Shell	1				
Soft tissue	0.725 ^a	1			
Sediment	− 0.899 ^{ab}	− 0.675 ^c	1		
Seawater	− 0.465	− 0.461	0.645	1	
Cu					
Shell	1				
Soft tissue	0.23	1			
Sediment	− 0.705 ^{ac}	0.027	1		
Seawater	− 0.505	− 0.84 ^{ab}	− 0.069	1	

^aStrong correlation at $r < -0.700$ or > 0.700

^bCorrelation is significant at the 99% level (2-tailed)

^cCorrelation is significant at the 95% level (2-tailed)

ability of *N. articulata* to reflect the Cu levels in their environment, especially the contamination in sediments. Besides, a significant positive correlation was observed between shells and seawater for Pb contamination ($r = 0.923$, $p < 0.01$). Moreover, a moderate and positive correlation was discovered between

seawater and soft tissue of *N. articulata* for Pb ($r = 0.608$), while negative correlations were found with Cd and Cu. Thus, combined with the BAF results in Table 7, the soft tissues of *N. articulata* are well-supported as potential bioindicators for Pb, which is consistent with the findings of Yap et al. (2009).

Regarding *C. obtusa*, negative correlations between soft tissues and sediment were observed with Pb and Cd, while a weak positive correlation was found with Cu (Table 9). However, between sediment and the shell, strong negative correlations were observed with Cd ($r = -0.899$, $p < 0.01$) and Cu ($r = -0.705$, $p < 0.05$). Besides, between soft tissue and seawater, strong and negative correlations were observed with Pb ($r = -0.715$, $p < 0.05$) and Cu ($r = -0.84$, $p < 0.01$) while a moderate correlation with Cd ($r = -0.461$).

In conjunction with the BSAF results in Table 7, the shell of *N. articulata* and the soft tissues of *C. obtusa* are suggested as potential biomonitoring indicators for Cd and Cu, respectively. Specifically, the shell of *N. articulata* is more suitable for monitoring the Pb and Cu contaminations in seawater. In contrast, the soft tissue indicates Cu contamination in sediment more accurately. For *C. obtusa*, the shell can monitor the Cd and Cu contaminations in sediment, whereas the soft tissue can be used to monitor the Pb and Cu contaminations in seawater.

Conclusion and future recommendations

In Kukup Fishing Village, the concentrations of Cd in both studied species exceeded previously reported levels, while the concentrations of Pb, Cu, and Cd also surpassed the limits established by the Malaysian Food Regulations (1985). Excess Pb may harm the brain, kidneys, and reproduction, while Cd damages kidneys and bones and increases cancer risk. This situation requires immediate intervention by relevant authorities, and the public is advised to reduce the consumption of sea snails from this area. Besides, from the local authority's perspective, efforts can be made on setting up policy to restrict the pass-by vessels' pollution to minimise possible oil leak issue. Moreover, local authorities can set up fundings to encourage scientists to explore the possibilities to solve the pollution issues. Regarding sediment analysis, all study locations exhibited

moderate to severe pollution, with particularly high Cd levels and relatively low Cu contamination. The elevated concentrations of these pollutants are likely associated with nearby human activities, such as construction waste, sewage discharge, coal combustion, and other related factors.

In terms of PTE accumulation, Pb is highly concentrated in the soft tissues of both species. Additionally, *C. obtusa* shows a greater capacity to accumulate Cd and Cu than *N. articulata*, particularly in the soft tissues of *C. obtusa*. This accumulation is more likely derived from sediments or the food chain rather than directly from seawater.

Considering the correlation between environmental conditions and the bioaccumulation in soft tissues and shells, the shell of *N. articulata* and the soft tissue of *C. obtusa* are considered effective bio-monitoring indicators for Cd and Cu, respectively. Specifically, the shell of *N. articulata* is more suitable for monitoring Pb and Cu pollution in seawater, while its soft tissue more accurately reflects Cu contamination from sediments. On the other hand, the shell of *C. obtusa* can be used to monitor Cd and Cu levels in sediments, while its soft tissue is more suitable for detecting Pb and Cu pollution in seawater.

However, this study was based on one-time sampling, which cannot reflect the dynamic trends of pollutant accumulation and lacks long-term monitoring data to assess the trends and risks associated with pollution accumulation. Moreover, the study was limited to a specific area of Kukup Fishing Village and solely focused on two species of sea snails (*N. articulata* and *C. obtusa*), with a relatively small sample size, which may not fully reflect pollution patterns across different seasons or broader areas. In the future, it is recommended to conduct multi-time, multi-season sampling to observe changes in PTE pollution over time and establish a long-term database to analyse the dynamic trends of PTE accumulation. Additionally, the sampling area and sample size should be expanded to include different seasons, tidal variations, and various water depths, thereby improving the representativeness and reliability of the study.

Author contribution RH Tan: conceptualization, methodology, investigation, data curation, writing an original draft. CC Ng: conceptualization, investigation, methodology, data curation, supervision, resources, funding requisition, reviewing and editing. T Gu: investigation, data curation, reviewing

and editing. PPY Tek: methodology, investigation, supervision, reviewing and editing.

Funding This study is financially supported by the Xiamen University Malaysia (XMUM) through the XMUMRF/2021-C7/ICAM/0008 grant.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval All authors have read, understood, and have complied as applicable with the statement on “Ethical responsibilities of Authors” as found in the Instructions for Authors. Moreover, it is important to note that both data availability and animal ethics declaration are not applicable in this context.

Competing interests The authors declare no competing interests.

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